J. exp. Biol. **107**, 147–161 (1983)
Printed in Great Britain © *The Company of Biologists Limited 1983*

SYMMETRICAL GIANT NEURONES IN ASYMMETRICAL GANGLIA: IMPLICATIONS FOR EVOLUTION OF THE NERVOUS SYSTEM IN PULMONATE MOLLUSCS

BY DOUGLAS P. MUNOZ*, PETER A. PAWSON† AND RONALD CHASE‡

Department of Biology, McGill University, 1205, Avenue Docteur Penfield, Montreal, Quebec, H3A 1B1, Canada

(*Received 11 January 1983–Accepted 18 March 1983*)

SUMMARY

Two of the largest neurones in the terrestrial snail *Achatina fulica* are RPr1, located in the right parietal ganglion, and V1, located in the visceral ganglion. Several characteristics of these cells were studied in detail, including morphology, passive and active electrical properties, synaptic inputs, sensory inputs, motor outputs and sensitivity to transmitter substances. The results suggest that RPr1 and V1 form a bilaterally homologous pair of cells, yet they reside in asymmetrically placed ganglia. An explanation of this paradox is offered in the proposal that RPr1 and V1 were formerly located in the bilaterally symmetrical intestinal ganglia. Their contemporary asymmetrical locations are accounted for by the hypothesis that, during the evolution of the pulmonate nervous system, the supraintestinal ganglion fused with the right pallial ganglion and the subintestinal ganglion fused with the visceral ganglion.

INTRODUCTION

The central nervous system of gastropod molluscs is composed of a collection of ganglia that have been subject to extensive changes during evolution. Comparative studies on the gross morphology of the nervous system in existing molluscan species, primitive and advanced, are helpful in reconstructing the history of ganglionic evolution (for reviews, see Bullock & Horridge, 1965; Kandel, 1979). A further source of information is the study of identifiable cells which are homologous in different molluscan species (Dorsett, 1974; Weiss & Kupfermann, 1976; Chase & Goodman, 1977; Dickinson, 1980). In these studies, it has been possible to infer common origins for homologous cells in hypothetical antecedent species. The concept of homologous cells can also be applied, in a somewhat different sense, to infer a common ganglionic origin for two cells which currently reside in different ganglia of the same, single species.

* Present address: Department of Neurophysiology, Montreal Neurological Institute, 3801 University St., Montreal, Quebec, H3A 2B4, Canada.

† Present address: UCLA School of Medicine, Jerry Lewis Neuromuscular Research Center, Los Angeles, CA, 90024, U.S.A.

‡ To whom reprint requests should be sent.

Key words: Homologous nerve cells, pulmonate mollusc, nervous system evolution.

An instructive example of an intraspecific homology that has been used to reconstruct evolutionary changes in ganglionic organization is the pair of neurones R2-LP1 in the marine gastropod, *Aplysia*. The giant neurone R2, in the abdominal ganglion, and the giant neurone LP1, in the pleural ganglion, presently reside in unpaired asymmetrical ganglia. However, the two neurones share many specific properties including axonal distribution (Hughes & Tauc, 1961), synaptic inputs (Hughes, 1967) and transmitter biochemistry (Giller & Schwartz, 1971). The marked similarity of R2 and LP1 led Hughes & Tauc (1963) to propose that the two neurones are an homologous pair which were once situated in the bilaterally symmetrical pallial ganglia. According to this view, the asymmetry in the cells' contemporary locations has occurred because of the asymmetrical fusion of the pallial ganglia with other ganglia. Specifically, the left pallial ganglion is thought to have fused with the left pleural ganglion, and the right pallial ganglion is thought to have fused with the supraintestinal ganglion to form the right half of the contemporary abdominal ganglion (Eales, 1921; Hughes & Tauc, 1963; Kandel, 1979). As a consequence of these changes, the somata of R2 and LP1 have assumed asymmetrical positions, while the distribution of the cells' axonal processes remains largely symmetrical.

In the pulmonate gastropod *Achatina fulica*, there exists a similar case of two giant neurones which closely resemble one another, yet are located in asymmetrical ganglia. The properties of these cells, RPr1 in the parietal ganglion and V1 in the visceral ganglion, are examined in detail in the present investigation. We have concluded that the neurones are an homologous pair whose contemporary locations can be accounted for by a history of asymmetrical fusion in earlier arrangements of the central nervous ganglia.

A preliminary report of some of these data has been published (Munoz, Pawson & Chase, 1982).

MATERIALS AND METHODS

Adult specimens of *Achatina fulica* (>150 days old), weighing 20–60 g, were taken from laboratory culture (P. A. Pawson & R. Chase, in preparation). The dissected tissue was pinned to a Sylgard base in a Petri dish and bathed in a saline solution which contained 67 mM-NaCl, 4 mM-KCl, 13 mM-$MgCl_2$, 11 mM-$CaCl_2$, 1 g l^{-1} glucose and 5 mM-Tris buffer. The pH was adjusted to 7·9 with HCl. For experiments in which it was necessary to block chemical transmission in the central ganglia, the ganglia were isolated from peripheral tissue with Vaseline partitions and perfused with a saline solution containing 26 mM-Mg and 0 mM-Ca.

Cells were penetrated with single- or double-barrel glass capillary electrodes filled with 2 M-K-citrate and bevelled to 5–10 MΩ resistance. Conventional electrophysiological recording and stimulating techniques were used (for details see Chase & Goodman, 1977; P. A. Pawson & R. Chase, in preparation). Muscle movements were recorded using a Grass FT.03 force displacement transducer. The transducer signal was amplified and filtered through a Grass P16 amplifier. Putative synaptic transmitter substances, in concentrations of 10^{-4} M, were dissolved in the high Mg saline (see above). These solutions were perfused through the bath at 6 ml min^{-1}. The bath capacity was about 7 ml.

Data were collected for RPr1 and V1 in each animal. Cell diameters were measured under a dissecting microscope using a micrometer eyepiece. The major (a) and minor (b) axes of the cell somata were measured and the equivalent diameters [$d = (a^2b)^{1/3}$] were calculated (Mirolli & Talbott, 1972). Current pulses were delivered through one side of a double-barrelled electrode, and voltage responses were recorded through the other side to determine rheobase current, threshold voltage, action potential amplitude, input resistance and time constant. Rheobase current was defined as the minimum amount of depolarizing current necessary to evoke a spike. The threshold voltage was defined as the difference between the resting membrane potential and the membrane potential at the time that an evoked spike was initiated. Action potential amplitudes were measured from resting potential to peak depolarization. The input resistance was calculated as the slope of the current-voltage curve in the linear portion of the hyperpolarizing response. Time constant values were derived from voltage transients that minimally displaced the cell membrane and fell in the linear portion of the cell's current-voltage curve (Gorman & Mirolli, 1972). The time constants were calculated as the negative reciprocal of the slope of $\ln(V_{max}-V_t)$ *versus* time (Merickel, Eyman & Kater, 1977). The data are presented throughout as means ± standard error (S.E.) of the mean.

Horseradish peroxidase (HRP: Sigma Type V1 or Boehringer Type I) was injected into cells through glass capillary electrodes using air pressure (7–14 kPa). The HRP was dissolved as a 2 % solution in 0·1 M-K-citrate. Filled cells were left for 2 h at room temperature or 24 h at 4 °C to allow diffusion of HRP into fine processes. The HRP was reacted with benzidine dihydrochloride and H_2O_2 according to histochemical procedures described by Muller & McMahan (1976). The ganglia containing cells filled with HRP were viewed as whole mounts. Peripheral tissue was cut on a cryostat as 60–90 μm sections following histological processing.

Lucifer Yellow CH (generously supplied by W. Stewart) was pressure injected into cells to test for dye coupling (Stewart, 1978). Electrode tips were filled with 1 % (w/v) Lucifer Yellow CH in 0·01 M-LiCl. Injected tissue was processed according to Stewart (1978) and viewed under a Leitz Orthoplan microscope equipped with incident excitation fluorescence.

RESULTS

Morphology

The two giant neurones, RPr1 and V1, are respectively located on the dorsal aspects of the right parietal and visceral ganglia (Fig. 1). They were readily identifiable on the basis of several criteria including the size and position of their somata, the action potential waveform and the nature of the spontaneous electrical activity.

RPr1 and V1 are among the largest cells in the nervous system of *Achatina fulica*. The equivalent soma diameter of RPr1 ranged from 194–345 μm while that of V1 ranged from 219–375 μm. Within a given animal the soma diameters were closely matched, with a mean difference of 24 ± 6 μm ($N = 16$). V1 was consistently larger than RPr1 (Chi-square, $P < 0{\cdot}05$).

When HRP is injected into RPr1 and V1 in the same animal, each cell appears as

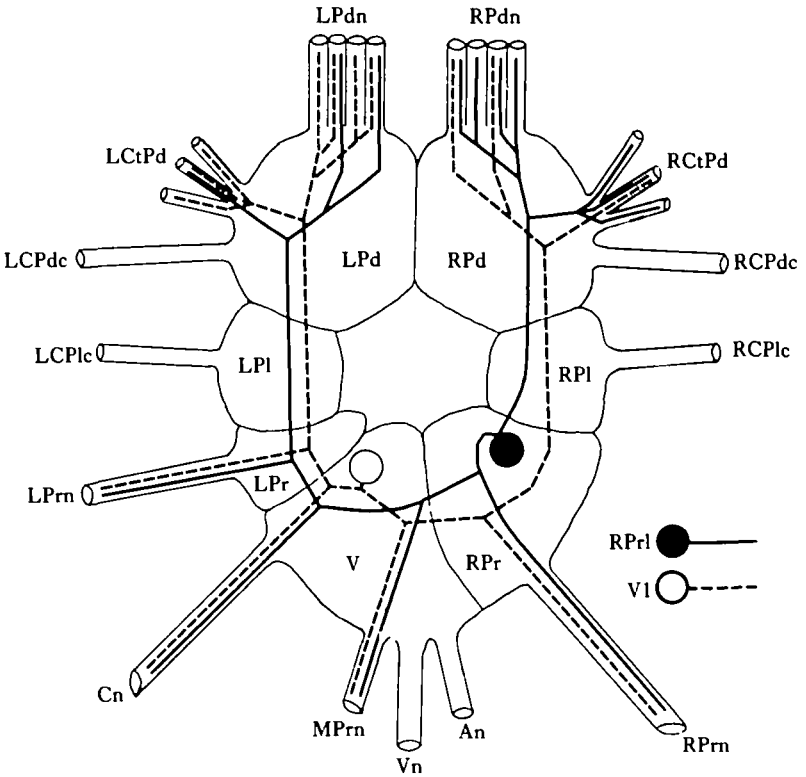

Fig. 1. Schematic representation of the dorsal aspect of the suboesophageal ganglionic ring (unfolded) in *Achatina fulica*. RPrl projections are represented by solid lines. Vl projections are represented by broken lines. The axons of each cell coincide in all nerves except the pedal nerves, where the ipsilateral cell is numerically favoured. The number and arrangement of the pedal nerves has been simplified for purposes of clarity. Abbreviations: Pd, pedal; Pl, pleural; Pr, parietal; V, visceral; An, anal nerve; Cn, columellar nerve; CPdc, cerebropedal connective; CPlc, cerebropleural connective, CtPd, cutaneous pedal nerves. Also: R, right; L, left; M, medial; n, nerve.

the mirror image of the other (Fig. 2A). Both cells have extensive axon branching in the right parietal, visceral and left parietal ganglia and somewhat reduced branching bilaterally in the pleural and pedal ganglia. Both cells send axons out the right parietal, medial parietal, columellar and left parietal nerves and have extensive projections in the multiple right and left pedal nerves. The pedal nerve projections heavily favour the ipsilateral side. Thus, both cells have many axon branches that project away from the suboesophageal ring, always in the posterior direction. Neither cell sends an axon into either pair of connective nerves linking the ring to the cerebral ganglia.

The peripheral destinations of HRP-filled axons were examined in serially sectioned material. The projections of RPrl and Vl in the pedal nerves travel to the foot musculature posterior to the pedal ganglia. The axons in the right parietal, medial parietal, columellar and left parietal nerves innervate the mantle region. In the case of the projections in the right parietal nerve, the axons of both RPrl and Vl were followed into mantle tissue of similar histological appearance where they branched and appeared to terminate (Fig. 2B, C).

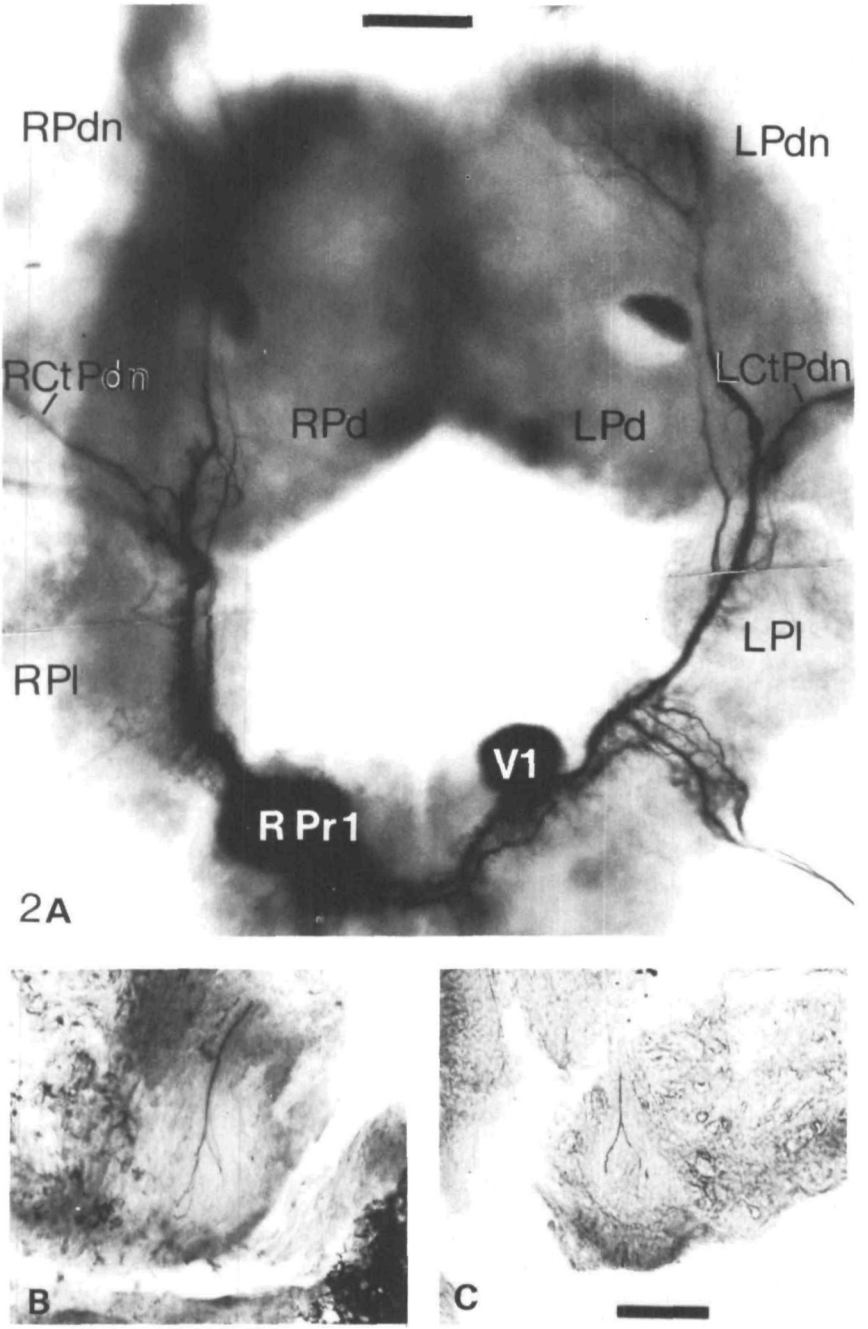

Fig. 2. (A) Morphological symmetry of RPr1 and V1 as revealed by intracellular injections of HRP. The figure is a composite of two photographs showing the ventral aspect of the suboesophageal ganglionic ring. Note the left–right reversal compared to Fig. 1. The statocysts are visible in the pedal ganglia. The thick process on each side belongs to the ipsilateral cell; the thin process belongs to the contralateral cell. Abbreviations as in Fig. 1. (B), (C) Sections through the mantle showing peripheral branches and presumed terminal sites of V1 axon (B) and RPr1 axon (C). (B) and (C) are taken from different animals. In both cases, the filled processes were followed from projections in the right parietal nerve. The magnification of (B) and (C) is the same. Scale bars: A = 300 μm; B, C = 100 μm.

Electrical properties

Neither cell produced spontaneous action potentials in the isolated preparation. The resting membrane potentials were closely matched. The resting potential of RPr1 was $-58{\cdot}3 \pm 1{\cdot}5$ mV ($N = 13$) and that of V1 was $-59{\cdot}0 \pm 1{\cdot}5$ mV ($N = 14$).

The passive membrane properties of RPr1 and V1 were remarkably similar. Fig. 3 shows typical passive responses to injection of current pulses as recorded from the two cells in a single animal. The charging transients (Fig. 3A, B) had a slow time course typical of molluscan giant neurones. The current-voltage curves (Fig. 3C) show that both cells exhibited anomalous rectification when hyperpolarizing responses were 15–20 mV below the resting membrane potential. The mean values for input resistance and time constant (Table 2) showed no significant difference in these parameters for the two cells (t-test; $P > 0{\cdot}05$).

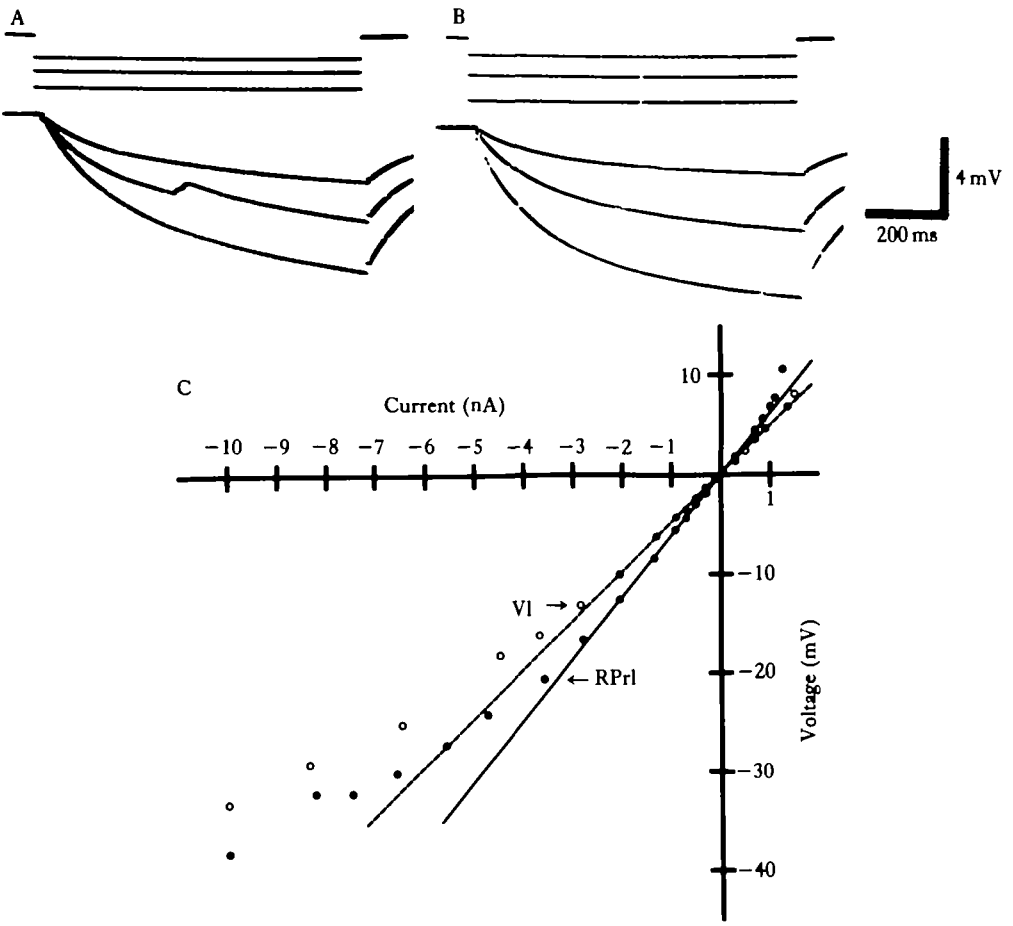

Fig. 3. Passive electrical properties. (A) and (B) show charging transients in RPr1 (part A) and V1 (part B) during current injection (top trace). (C) Current-voltage relationships for RPr1 (filled circles) and V1 (open circles). Current pulses were 2 s in duration. The linear part of the hyperpolarizing portion of the curve was used to determine the cells' input resistances (R_m). Here, R_{in} (RPr1) = 6·14 MΩ; R_{in} (V1) = 4·90 MΩ. Note that both cells show anomalous rectification.

The electrical excitability of RPr1 and V1 was also similar. Measurements of rheobase current, threshold voltage and action potential amplitude (Table 2) showed no significant difference between the cells (t-test; $P > 0{\cdot}05$). Both cells had prominent negative afterpotentials of 15–20 mV following a spike (Figs 7, 8).

Inputs

There was a high degree of synchrony in the spontaneously occurring EPSPs

Fig. 4. Spontaneous synaptic inputs recorded simultaneously in RPr1 and V1. With few exceptions, EPSPs are synchronous in the two cells.

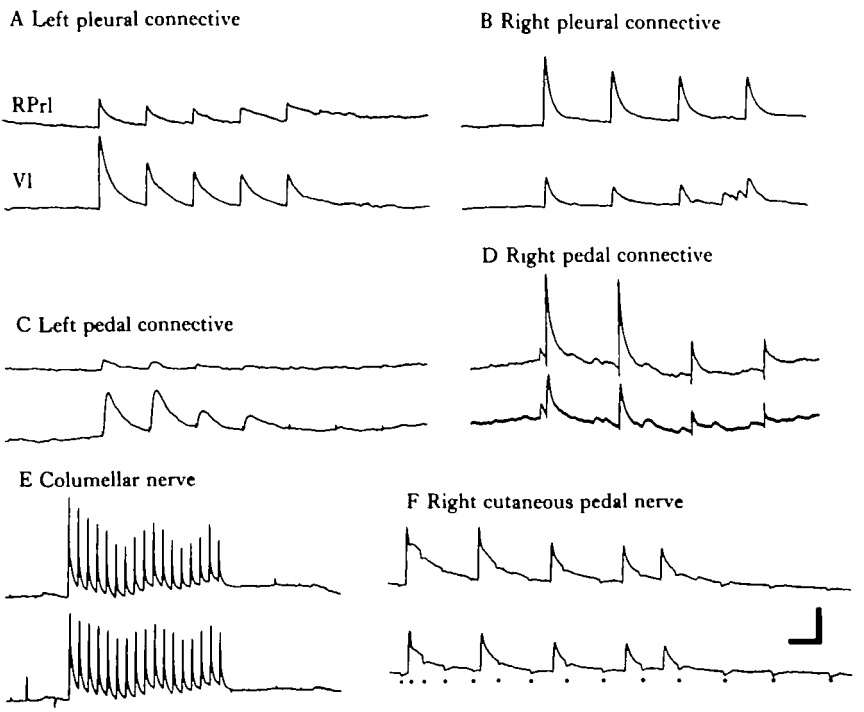

Fig. 5. Electrical stimulation of nerves and connectives. Repeated pulses (0·5 ms) were delivered through suction electrodes. Cellular responses are compound EPSPs. Note that when homologous connectives were stimulated independently on the right and left sides (A, B and C, D), the cell ipsilateral to the site of stimulation consistently produced the largest response. Depression of the response occurs in all cases. (E) Slow time scale shows that trial-to-trial variations in relative EPSP amplitude are closely matched in the two cells. (F) Synchronous IPSPs (marked by dots), as well as EPSPs, following stimulation of a cutaneous pedal nerve. IPSPs continue after termination of stimulus. Calibration scales are: A–D, 3 mV, 2 s; E, 7·5 mV, 10 s; F, 7·5 mV, 2 s.

Table 1. *Temporal order of spiking response to mechanical stimulation of the skin*

(A) Stimulus to the left side ($N = 12$)	
V1 precedes RPr1	12
RPr1 precedes V1	0
(B) Stimulus to the right side ($N = 13$)	
V1 precedes RPr1	1
RPr1 precedes V1	12

The dorso-lateral margin of the foot was gently stroked with a wooden probe. The ipsilateral neurone reaches threshold before the contralateral neurone.

recorded in RPr1 and V1 (Fig. 4). Spontaneous IPSPs were rare, but those evoked by nerve stimulation were likewise synchronous (Fig. 5F). The EPSPs tended to be larger in RPr1 than in V1 (Fig. 4), probably owing to the larger soma size of V1 which could result in a greater electrotonic attenuation of synaptic potentials propagating to the somatic electrode from synaptic sites in the neuropil.

Electrical stimulation of any of the cerebropleural or cerebropedal connective nerves led to synchronous, compound EPSPs in RPr1 and V1 (Fig. 5A–D). The response was always larger in the cell ipsilateral to the stimulated connective. A lateral bias was also seen in the latency of response following mechanical stimulation of the foot. The cell located ipsilateral to the site of stimulation consistently reached threshold before the contralateral cell (Table 1).

Electrical stimulation of any of the peripheral nerves joining the suboesophageal ganglion also led to synchronous, compound EPSPs. Repeated nerve stimulation resulted in depression of the compound EPSP (Fig. 5). The rate of depression varied as a function of the nerve which was being stimulated and the recent history of stimulation, but the course of depression was always parallel in the two cells. Even trial to trial variations in response amplitude were closely matched (Fig. 5E). Stimulation of the cutaneous pedal nerves led to synchronous IPSPs in RPr1 and V1, as well as depressing compound EPSPs (Fig. 5F).

The giant neurone V2, located in the visceral ganglion, is a source of monosynaptic input to RPr1 and V1 (P. A. Pawson & R. Chase, in preparation). Intrasomatic stimulation of this neurone evoked synchronous, facilitating EPSPs in both postsynaptic cells (Fig. 6). The time course of facilitation was very similar.

A possible explanation for the synchrony of electrical events in RPr1 and V1 might be that the two cells are electrically coupled. Our experiments, however, do not support such an interpretation. Current injection into either cell produced no detectable change of membrane potential in the other cell. Nor were there coupling potentials associated with spiking activity. Finally, no dye coupling was observed following injection of Lucifer Yellow into either cell. Together, these results argue against the presence of electrical coupling between RPr1 and V1 (Stewart, 1978).

The preponderant occurrence of synchronous synaptic potentials in RPr1 and V1 therefore implies that the majority of inputs to each cell derive from presynaptic neurones which also provide inputs to the other cell, i.e. RPr1 and V1 are parallel pathways of information transfer.

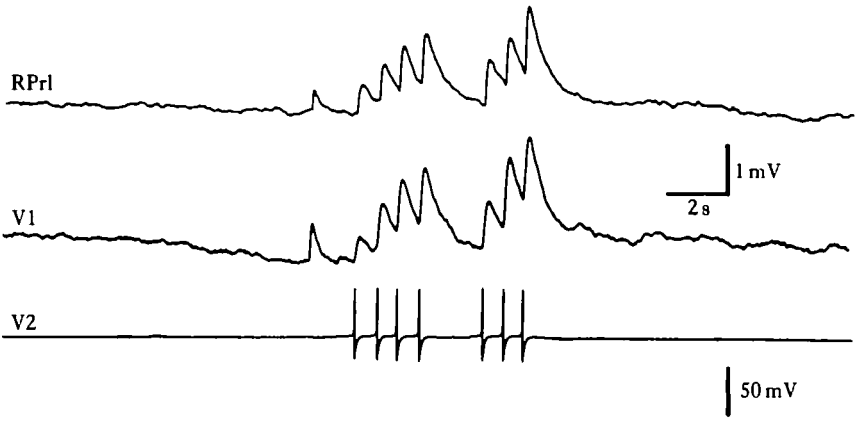

Fig. 6. Intrasomatic stimulation of cell V2 leads to parallel facilitation of EPSPs in RPrl and V1. High frequency filtration was employed in recording the postsynaptic responses.

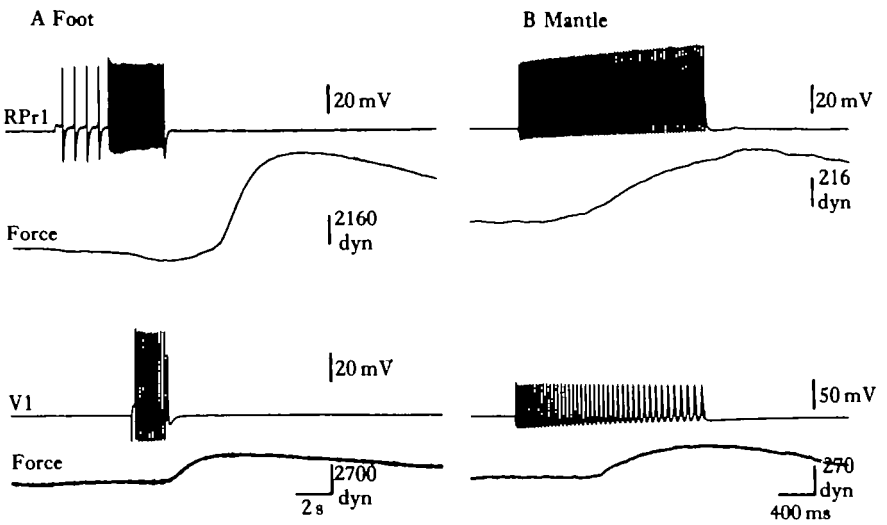

Fig. 7. Peripheral motor responses driven by intracellular stimulation. Muscular contractions were recorded with a force transducer. In (A), the central nervous system was bathed in a Ringer solution containing elevated levels of Mg^{2+} (twice normal) and zero Ca^{2+}. The effectiveness of the Ringer in blocking chemical synaptic transmission was judged by the disappearance of spontaneous synaptic potentials (see Fig. 10) and by the disappearance of responses evoked by peripheral mechanical stimulation.

Outputs

Intrasomatic electrical stimulation of either RPrl or V1 resulted in movements of the foot (Fig. 7A) and the mantle (Fig. 7B). Strong muscular contractions of the foot could be evoked even when chemical synaptic transmission in the central ganglia was suppressed by the use of a Ringer solution containing twice the normal Mg^{2+} concentration and zero Ca^{2+} (Fig. 7A). Since injections of Lucifer Yellow revealed no transneuronal migration of dye, and thus presumably no electrical synapses with

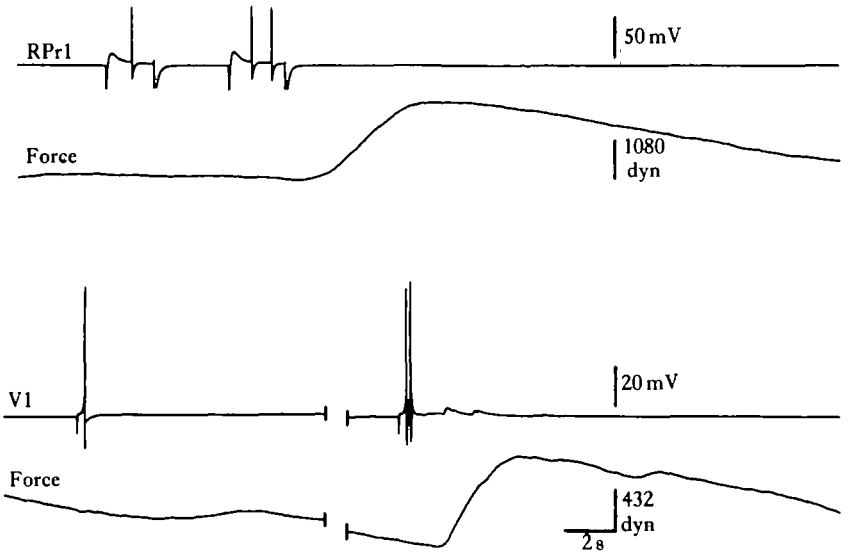

Fig. 8. Facilitation of the behavioural response, measured as contractions of the foot. A single spike produces no detectable response, but two spikes at a short interval produce a large response.

follower cells (Stewart, 1978), the evidence suggests that both RPr1 and V1 are responsible for taking a motor command to the periphery.

Repeated activity in RPr1 or V1 led to short-term facilitation and long-term depression of the behavioural response. Facilitation was demonstrated by the fact that a single spike in either cell was ineffective in producing any observable movement, whereas two spikes separated by an interval of 1 s or less evoked a pronounced response (Fig. 8). Depression was seen with repeated bursts of spikes elicited at 1 min^{-1}. Repeated stimulation led to a progressive decrease in response strength (Fig. 9A) accompanied by an increase in response latency (Fig. 9B).

Responses to transmitters

Substances previously identified as synaptic transmitters in molluscan ganglia were added to the saline perfusion while recording simultaneously from RPr1 and V1. In every case, the two cells responded in a qualitatively similar manner to the introduced chemicals. Acetylcholine produced a synchronous hyperpolarization (Fig. 10A). Addition of serotonin, GABA, or octopamine led to a depolarization (Fig. 10B, C, D). Neither glycine nor glutamic acid had any detectable effect (Fig. 10E, F) at the concentration used.

DISCUSSION

We have described a remarkable similarity in the properties of two molluscan neurones. The large number of properties which are shared by the two cells (summarized in Table 2) is unlikely to be fortuitous, especially since some of these properties, e.g. soma size, HRP morphology and motor drive to peripheral muscle, are unusual or unique.

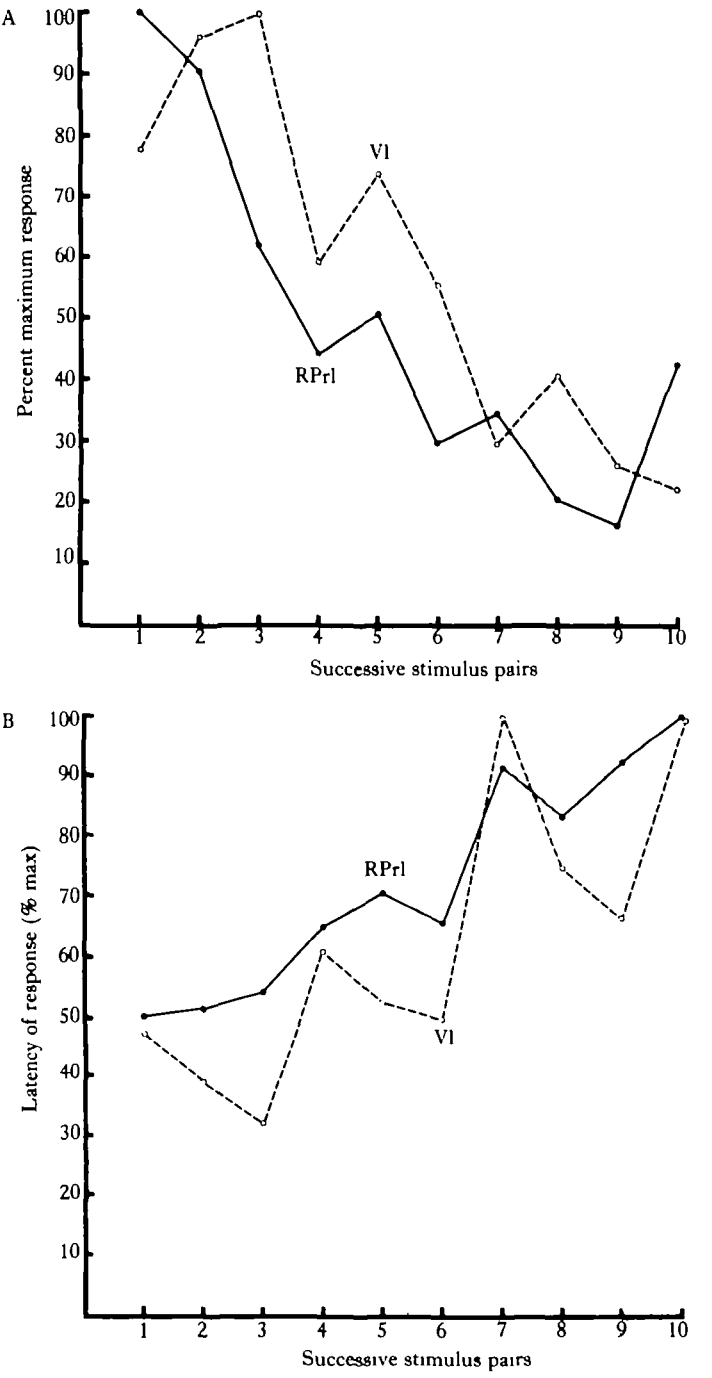

Fig. 9. Depression of the behavioural response. In separate experiments, RPrl (solid line) and VI (broken line) were stimulated intracellularly with depolarizing pulses (2 s) at 1 min^{-1}. For RPrl, the number of spikes elicited by each stimulus was constant within the range 8–10; for VI, the number of spikes per stimulus increased about 30 % during the series of trials. The graphs show data which has been pooled from successive stimulus pairs. (A) Amplitude of foot contractions. (B) Latency of foot contractions measured from the first spike in the evoked train of spikes.

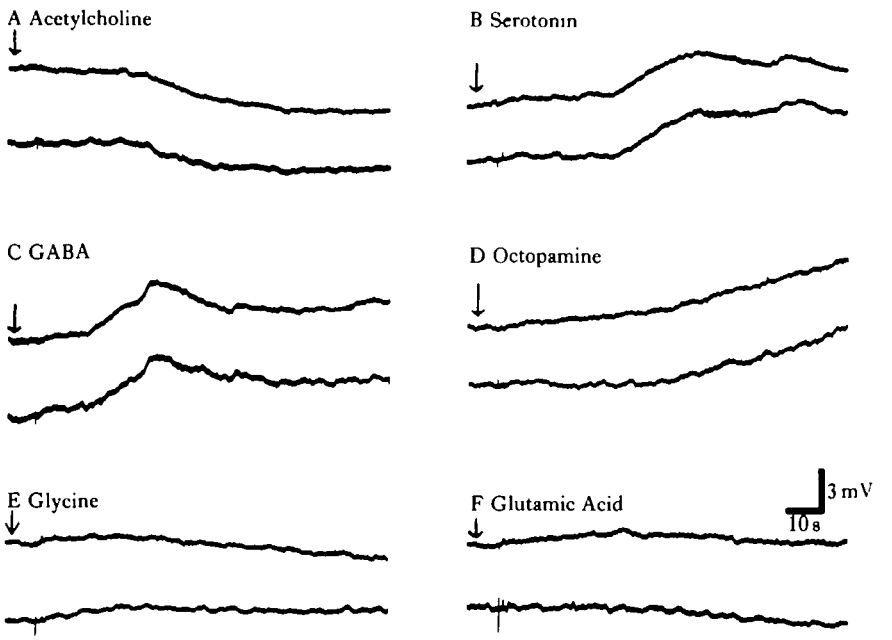

Fig. 10. Responses to putative transmitter substances. Transmitter chemicals, in concentration of 10^{-4} M, were perfused at 6 ml min^{-1} beginning at the arrow. The Ringer in these experiments contained Mg^{2+} at twice normal levels and Ca^{2+} at zero concentration. Acetylcholine hyperpolarizes RPr1 and V1, while serotonin, GABA and octopamine depolarize. Glycine and glutamic acid have no effect.

While the properties of RPr1 and V1 are, for the most part, very similar, they also differ in a consistent manner with respect to the laterality of their inputs and outputs. The relevant data are the following: (1) electrical stimulation of the cerebral connectives evokes compound EPSPs in both cells, but the response in the ipsilateral cell is always larger than in the contralateral cell (Fig. 5A–D), (2) mechanical stimulation of the foot causes the ipsilateral cell to reach threshold before the contralateral cell (Table 1) and (3) the axon collaterals of each cell favour the ipsilateral side of the body (Figs 1, 2). Together, these findings suggest that RPr1 and V1 might be considered as a bilaterally homologous pair of atypically large neurones similar to the giant metacerebral cells in gastropod molluscs (Kandel & Tauc, 1966; Weiss & Kupfermann, 1976), the Retzius cells in leech abdominal ganglia (Lent, 1973), or the Mauthner cells in fish and amphibian medulla (Faber & Korn, 1978). Seemingly at odds with this interpretation, however, is the fact that RPr1 and V1 have cell bodies which are located in two ganglia which are not bilaterally symmetrical.

The present pair of cells is similar to the pair R2-LP1 in *Aplysia*, because in both cases symmetrical cellular properties co-exist with asymmetrical locations. Hughes & Tauc (1963) attempted to explain this paradox in *Aplysia* by proposing that the two cell bodies were originally located in symmetrically paired pallial ganglia at an ancestral stage in the evolution of the species. An analogous argument can be made in the case of the neurones RPr1 and V1 in *Achatina*.

Table 2. *Summary of properties of the neurones RPr1 and V1*

Property	RPr1	V1
Soma diameter	267 ± 12 μm (N = 16)	286 ± 14 μm (N = 16)
Nerve projections (abbreviations explained in Fig. 1)	RPrn, MPrn, Cn, LPrn, Pdn (ipsilateral bias)	RPrn, MPrn, Cn, LPrn, Pdn (ipsilateral bias)
Resting membrane potential	− 58·3 ± 1·5 mV (N = 13)	− 59·0 ± 1·5 mV (N = 14)
Rectification	Anomalous	Anomalous
Input resistance	5·2 ± 0·4 MΩ (N = 11)	4·6 ± 0·5 MΩ (N = 12)
Time constant	401 ± 38 ms (N = 10)	501 ± 51 ms (N = 8)
Rheobase current	2·56 ± 0·34 nA (N = 10)	2·25 ± 0·23 nA (N = 9)
Threshold voltage	17·4 ± 1·3 mV (N = 10)	19·3 ± 1·4 mV (N = 8)
Action potential amplitude	90·2 ± 2·4 mV (N = 11)	89·2 ± 2·8 mV (N = 10)
Spontaneous activity	EPSPs; no spikes	EPSPs; no spikes
Inputs		
Body skin	Mechanosensory excitation	Mechanosensory excitation
All connectives and peripheral nerves	Depressing compound EPSPs	Depressing compound EPSPs
Cutaneous pedal nerves	IPSPs, EPSPs	IPSPs, EPSPs
Neurone V2	Facilitating EPSPs	Facilitating EPSPs
Motor outputs	Mantle	Mantle
	Foot (short-term facilitation; long term depression)	Foot (short-term facilitation; long term depression)
Response to transmitters		
Acetylcholine	Hyperpolarization	Hyperpolariazation
Serotonin	Depolarization	Depolarization
GABA	Depolarization	Depolarization
Octopamine	Depolarization	Depolarization
Glycine	No response	No response
Glutamic acid	No response	No response

The major scientific problem in the evolution of the gastropod nervous system is to explain why the condition of chiastoneury (crossing of the visceral connectives) is present in all members of the most primitive subclass, the Prosobranchia, and in some of the Opisthobranchia, but not in the Pulmonata (with the exception of *Chilina*, see below). While it is generally believed that the characteristic euthyneury (uncrossed arrangement) of the opisthobranchs resulted from a process of detorsion coincident upon a posterior migration of the mantle complex, euthyneury in the pulmonates does not appear to have followed detorsion (for reviews, see Bullock & Horridge, 1965; Kandel, 1979). Rather, in the pulmonates there was apparently a drastic shortening of the connectives and a fusion of ganglia (Bargmann, 1930). There are wide disagreements in the literature, however, as to the details of this inferred historical process. The interpretation which we provide below is based on a selection and coalescence of earlier ideas, consistent with the experimental results described in the present paper.

The nervous system of the hypothetical prosobranch ancestor to modern pulmonates was characterized, in part, by a circumoesophageal ring of 'head ganglia' comprising the bilaterally paired pleural, pedal and cerebral ganglia. Chiastoneury was present, with the supraintestinal ganglion on the left side of the body and the subintestinal ganglion on the right side (Fig. 11A). The most posterior portion of the body was innervated by the unpaired visceral ganglion. As the visceral nerve loop elongated, two new ganglia appeared (Brace, 1977). These new ganglia, termed pallial because they innervate the mantle, are present in forms which are considered transitional between

the prosobranchs and the pulmonates (Fig. 11B). Subsequently, there occurred a shortening of the connectives that brought the supraintestinal ganglion to the right side and the subintestinal ganglion to the left side. At the same time, shortening of the connectives led to a fusion of ganglia. The primitive pulmonate *Chilina* (Fig. 11C) represents a presumed transitional stage in this process (Haeckel, 1913). In *Chilina*, the supraintestinal ganglion has fused with the right pallial ganglion to form the bilobed right parietal ganglion. The subintestinal ganglion has moved close to the visceral ganglion but has not yet fused with it. In the final stage of pulmonate evolution, attained by the stylommatophoran pulmonates such as *Achatina* (Fig. 11D), a further shortening of the connectives resulted in the fusion of the subintestinal and visceral ganglia (Pelseneer, 1901; Krull, 1934). Thus, of the five posterior ganglia of early pulmonates, only the left pallial ganglion (now called left parietal) has not undergone fusion.

Of particular interest in the above account is the assumption that the two intestinal ganglia fused asymmetrically. While the supraintestinal ganglion fused with the pallial

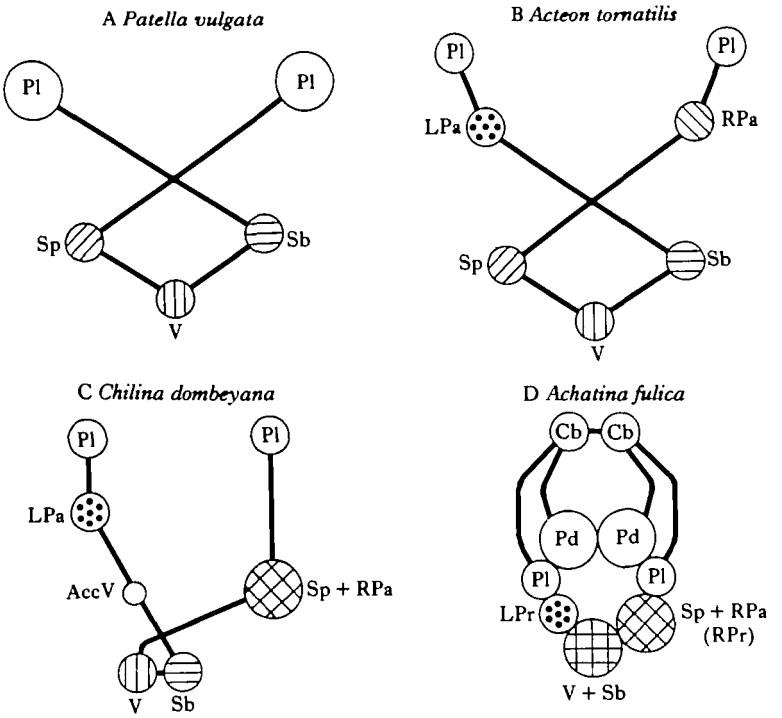

Fig. 11. Organization of the central nervous system (posterior portion) in gastropod molluscs. The examples show contemporary species that are considered (by the cited authors) to represent successive stages in the evolution of the pulmonate nervous system. (A) A prosobranch gastropod (Bargmann, 1930). (B) A primitive opisthobranch (Brace, 1977). (C) A primitive pulmonate (Haeckel, 1913). (D) An advanced pulmonate and the subject of the present study. The fusion of ganglia is indicated by cross-hatching. Abbreviations: AccV, accessory visceral ganglion; Cb, cerebral ganglion; Pa, pallial ganglion; Pd, pedal ganglion; Pl, pleural ganglion; Pr, parietal ganglion; Sb, subintestinal ganglion; Sp, supraintestinal ganglion; V, visceral ganglion; L, left; R, right.

ganglion on the right, the subintestinal ganglion fused with the visceral ganglion in the centre. It is our contention that the similarity of the neurones RPr1 and V1 is a vestige of an earlier bilateral symmetry in the locations of the sub- and supraintestinal ganglia. The cells can be called an homologous pair because RPr1 was originally located in the supraintestinal ganglion and V1 was located in the subintestinal ganglion. While this argument is speculative, it is nonetheless consistent with certain previous interpretations of the evolution of the pulmonate nervous system (Bargmann, 1930; Krull, 1934; Brace, 1977). Our data on identifiable cells therefore support the evolutionary view presented, which previously has been supported only by observations on the geometric arrangement of the ganglia and the innervation of the ganglia by peripheral nerves.

This work was supported by a grant given to R. Chase from the Natural Sciences and Engineering Research Council of Canada.

REFERENCES

BARGMANN, H. E. (1930). The morphology of the central nervous system in the Gastropoda Pulmonata. *J. Linn. Soc. (Zool)* **37**, 1–70.

BRACE, R. C. (1977). Anatomical changes in nervous and vascular systems during the transition from prosobranch to opisthobranch organization. *Trans. zool. Soc. Lond.* **34**, 1–25.

BULLOCK, T. H. & HORRIDGE, G. A. (1965). *Structure and Function in the Nervous System of Invertebrates*, Vol. II. San Francisco: W. H. Freeman.

CHASE, R. & GOODMAN, H. (1977). Homologous neurosecretory cell groups in the land snail *Achatina fulica* and the sea slug *Aplysia californica*. *Cell Tiss. Res.* **176**, 109–120.

DICKINSON, P. S. (1980). Neuronal control of gills in diverse *Aplysia* species: Conservative evolution. *J. comp. Physiol.* **139**, 17–23.

DORSETT, D. A. (1974). Neuronal homologies and the control of branchial tuft movements in two species of *Tritonia*. *J. exp. Biol.* **61**, 639–654.

EALES, N. B. (1921). *Aplysia*. Liverpool Marine Biology Committee Memoirs. 24, *Proc. Trans., Liverpool biol. Soc.* **35**, 183–266.

FABER, D. & KORN, H. (eds) (1978). *Neurobiology of the Mauthner Cell*. New York: Raven Press.

GILLER, E. & SCHWARTZ, J. H. (1971). Choline acetyltransferase in identified neurons of abdominal ganglion of *Aplysia californica*. *J. Neurophysiol.* **34**, 93–107.

GORMAN, A. L. F. & MIROLLI, M. (1972). The passive electrical properties of the membrane of a molluscan neurone. *J. Physiol., Lond.* **227**, 35–49.

HAECKEL, W. (1913). Beiträge zur anatomie der gattung *Chilina*. *Zool. Jb. Suppl.* **13**, 89–136.

HUGHES, G. M. (1967). Further studies on the electrophysiological anatomy of the left and right giant cells in *Aplysia*. *J. exp. Biol.* **46**, 169–193.

HUGHES, G. M. & TAUC, L. (1961). The path of the giant cell axons in *Aplysia depilans*. *Nature, Lond.* **191**, 404–405.

HUGHES, G. M. & TAUC, L. (1963). An electrophysiological study of the anatomical relations of two giant nerve cells in *Aplysia depilans*. *J. exp. Biol.* **40**, 469–486.

KANDEL, E. R. (1979). *Behavioral Biology of Aplysia*. San Francisco: W. M. Freeman.

KANDEL, E. R. & TAUC, L. (1966). Input organization of two symmetrical giant cells in snail brain. *J. Physiol., Lond.* **183**, 269–286.

KRULL, H. (1934). Die Aufhebung der Chiastoneurie bei den Pulmonaten. *Zool. Anz.* **105**, 173–182.

LENT, C. M. (1973). Retzius cells from segmental ganglia of four species of leech: comparative neuronal geometry. *Comp. Biochem. Physiol.* **44**, 35–40.

MERICKEL, M. B., EYMAN, E. D. & KATER, S. B. (1977). Analysis of a network of electrically coupled neurons producing rhythmic activity in the snail *Melisoma trivolvis*. *I.E.E.E. Trans. Biomed. Eng.* **24**, 277–287.

MIROLLI, M. & TALBOTT, S. R. (1972). The geometrical factors determining the electrotonic properties of a molluscan neurone. *J. Physiol., Lond.* **227**, 19–34.

MULLER, K. J. & MCMAHAN, U. J. (1976). The shapes of sensory and motor neurones and the distribution of their synapses in ganglia of the leech: A study using intracellular injection of horseradish peroxidase. *Proc. R. Soc. B.* **194**, 481–499.

MUNOZ, D., PAWSON, P. A. & CHASE, R. (1982). Symmetrical giant neurons in asymmetrical molluscan ganglia. *Soc. Neurosci. Abstr.* **8**, 715.

'ELSENEER, P. (1901). Études sur des gastropodes pulmonés. *Mém. Inst. r. Sci. nat. Belg.* **54**, 1–76.

'TEWART, W. W. (1978). Functional connections between cells as revealed by dye-coupling with a highly fluorescent naphthalimide tracer. *Cell* **14**, 741–759.

VEISS, K. R. & KUPFERMANN, I. (1976). Homology of the giant serotonergic neurons (metacerebral cells) in *Aplysia* and pulmonate molluscs. *Brain Res.* **117**, 33–49.